Combined Heat and Controlled Atmosphere Quarantine Treatments for Control of Codling Moth in Sweet Cherries

LISA G. NEVEN

Yakima Agricultural Research Laboratory, USDA–ARS, 5230 Konnowac Pass Road, Wapato, WA 98951

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ABSTRACT Nonchemical quarantine treatments, using a combination of short-duration high temperatures under low oxygen, elevated carbon dioxide atmospheric environment were developed to control codling moth in sweet cherries, *Prunus avium* (L.). The two treatments developed are a chamber temperature of 45°C for 45 min and a chamber temperature of 47°C for 25 min under a 1% oxygen, 15% carbon dioxide, −2°C dew point environment. Both these treatments have been shown to provide control of all life stages of codling moth while preserving commodity market quality. The third and fourth instars of codling moth are equally tolerant to CATTS treatments and are the most tolerant immature stages to these treatments. We determined that low levels of oxygen are more important than elevated carbon dioxide in achieving high levels of insect mortality. Efficacy tests of both treatments resulted in 100% mortality of 5,000 third instars of codling moth in each treatment. These treatments may be used to provide quarantine security in exported sweet cherries where codling moth is a quarantine concern and fumigation with methyl bromide is not desired.

KEY WORDS *Cydia pomonella*, quarantine, cherry, controlled atmosphere

CODLING MOTH, *Cydia pomonella* (L.), is the principal pest of quarantine concern in sweet cherries exported from the Pacific Northwest to Japan and Taiwan (http://www.nwhort.org/japan.html). Currently, sweet cherries, *Prunus avium* (L.), are fumigated with methyl bromide to ensure quarantine security (FAO 1983; Moffitt et al. 1992; NWHC 2004a, b). However, methyl bromide fumigation is not in compliance with U.S. and Japanese established organic standards (MAFF 2000, USDA–AMS 2004). Development of organic compliant quarantine treatments for organic sweet cherries would aid in the economic development of organic producers in the United States.

It is generally recognized that plants and fruits have a relatively high capacity for anaerobic metabolism. The most prevalent by-product of plant anaerobic metabolism is ethanol, which is highly volatile. Insects, however, have a very limited capacity for anaerobic metabolism. The presence of oxygen is critical for insect thermal acclimation to thermal stress (Yocum and Denlinger 1994). In addition, insect respiration is chiefly regulated by the presence of carbon dioxide (Buck 1962, Kestler 1985) (primarily for terrestrial arthropods, but not including certain semiaquatic insects such as tephritid larvae.) Using this difference in metabolic response to stress, both thermal and respiratory, has led to the development of high temperature treatments under low oxygen and elevated carbon dioxide atmospheres.

The Controlled Atmosphere Temperature Treatment System, CATTS (Neven and Mitcham 1996), combines the application of forced moist or vapor hot air under a controlled atmosphere (CA) (i.e., low oxygen, elevated carbon dioxide). This technology is similar to existing vapor and forced hot air treatment systems currently approved and in use for several commodities entering many countries worldwide (Hallman and Armstrong 1994, USDA–APHIS–PPQ 2003). The difference in CATTS is the application of a controlled atmosphere. Controlled atmosphere treatments reduce the time necessary for 100% kill of the pest compared with heat treatment alone, and by decreasing the duration of the treatment, a combination treatment of this type can often reduce any adverse effects on fruit quality caused by heat treatment (Yahia 2000a, b, c; Neven et al. 2001; Shellie et al. 2001). Combination heat and controlled atmosphere treatment research has increased in recent years (Neven and Mitcham 1996; Shellie et al. 1997; Whiting and Hoy 1997; Neven and Drake 1998, 2000; Whiting et al. 1999; Yahia 2000a, b, c; Neven et al. 2001; Shellie et al. 2001; Heather et al. 2002; Neven 2004).

This article focuses on the identification of the most tolerant stage of codling moth infesting sweet cherries to moist, hot forced air treatments under low oxygen and elevated carbon dioxide atmospheres, and development of a treatment protocol to achieve postharvest control of this pest. This study also examines the effect

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of various levels of oxygen and carbon dioxide on codling moth mortality.

Materials and Methods

Most Tolerant Stage. Approximately 250 pairs of codling moths were placed into a wax paper bag and held for 24 h at 23°C, 70% RH, and a photoperiod of 18:6 (L:D) h for oviposition. The moths were then removed from the wax bag and discarded. Eggs in the paper bags were held for various periods of time to obtain three stages of eggs (white [0–2 d], red ring [2–3 d], and blackhead [4–7d]). Eggs of the appropriate stage were cut from the wax bag and placed on to mature 'Bing' and 'Rainier' sweet cherries by using a small droplet of low melt hot glue (Magic Melt Floral Glue, Adhesive Technologies, Inc., Hampton, NH), to prevent removal during treatment by the circulation fan in the CATTS unit. Groups of 200 eggs were treated at each time and temperature combination, and each combination was repeated at least four times. Control eggs, 200 for each date the tests were run, were similarly placed on cherries but not subjected to CATTS treatment. The fruit were treated immediately after the placement of the eggs on the surface, to ensure eggs did not develop further. After CATTS treatment, treated and control eggs were subjected to hydrocooling for 5 min at 0°C. Treated and control eggs on fruit were held at normal rearing conditions (23°C, 70% RH and a photoperiod of 18:6 [L:D] h) for 7 d, and both control and treated eggs on fruit were examined for egg hatch.

Codling moth larvae were reared on artificial diet (Toba and Howell 1991) at 23°C, 70% RH, and a photoperiod of 18:6 (L:D) h. Groups of 50 larvae were counted and placed on to 50 mature Bing and Rainier sweet cherries. First through third instars were transferred from artificial diet using a size 00 camel's-hair brush, and fourth and fifth instars were transferred from the diet by using soft forceps. A group of 50 larvae on 50 fruits served as untreated controls for each day of testing. Infested fruits were held at normal rearing conditions (23°C, 70% RH and a photoperiod of 18:6 [L:D] h) for 1 d before treatment. Only insects in the fruit at treatment time were counted in the treatment total. Each time and temperature combination treatment was repeated at least four times. After CATTS treatment, treated and control larvae were subjected to hydrocooling for 5 min at 0°C. Treated and control larvae in fruit were held at normal rearing conditions (23°C, 70% RH, and a photoperiod of 18:6 [L:D] h) for 1 d, and then fruit were cut or split open and examined for the presence of larvae. Larvae were scored as live, dead, or moribund. Moribund larvae were placed on organically produced thinning apples, because cherries are a poor host and do not last for 7 d at room temperatures, and held under normal rearing conditions (23°C, 70% RH, and a photoperiod of 18:6 h [L:D]) for 7 d, after which the fruit were cut open and larvae were scored as being either live or dead. All CATTS treatments used in these studies were not optimized for achieving core target temperatures (For the 45°C CATTS treatment core temperature reaching 42°C within 7–9 min with final core temperature of 44.5°C in 22–24 min. For the 47°C CATTS treatment fruit core temperatures reaching 42°C within 7–9 min and final core temperature of 45.5°C in 12–14 min), as was the case for the efficacy trials.

CATTS Treatments. The CATTS chamber (Techni-Systems, Chelan, WA) used in these studies has been described previously (Neven and Mitcham 1996). Treatment lugs used in these tests were standard vented bottom OnoPac (Hilo, HI) papaya treatment lugs (38.1 by 53.3 by 15.2 cm). The bottom of the lugs was lined with nylon organdy that was secured in place with hot glue and duct tape. A layer of nylon organdy also was placed on the top of the lugs and secured in place with double stick tape and/or hot glue to prevent larvae from dropping out of the lug during treatment. Infested fruit were placed into the lugs, and three temperature probes were placed randomly under one surface of a fruit and into two fruit cores. The treatment chamber was run until treatment conditions for the test were achieved before loading the infested fruit. Treatment 1 consisted of a chamber temperature of 45°C, under 1% oxygen, 15% carbon dioxide environment with the dew point set to 2°C below the fruit surface temperature. Air speed was set at 2 m/s. Treatment #2 consisted of a chamber temperature of 47°C, with all other conditions the same as for treatment #1. Lugs of infested fruit were placed into the lug exchanger, which was part of the original CATTS system, and the lug exchanger was attached to the frame of the CATTS chamber. The lug exchanger was designed be attached to the front of the CATTS chamber to minimize loss of atmospheric conditions during loading. The lug exchanger is a 156.85 by 73.66 by 45.72 cm (height by width by depth) unit that has four chambers (24.13 by 60.96 by 44.45 cm) that hold the treatment lugs and line up with the four doors of the CATTS chamber. The unit attaches to the front of the CATTS chamber with quick release clamps. Compressible neoprene gaskets seal around the inner door frame unit that contains the sliding doors used for lug insertion. The lug exchanger was flushed with nitrogen for 2 min before inserting the lug into the chamber to help maintain CA levels in the CATTS chamber during exchanges. A sliding door was opened on the chamber and the lug inserted into the chamber. At that time, the temperature probe connector was attached to the junction inside the chamber to allow for monitoring of treatment temperatures. The door was slid shut immediately. Exchange of lugs normally took between 8 and 15 s. After the insertion of the lug into the chamber, the lug exchanger was detached from the chamber and the outer door sealed. The reverse process was used to retrieve lugs after treatment. All treated and control infested fruits were subjected to hydrocooling for 5 min at 0°C.

Large-Scale Efficacy Tests. Efficacy tests against 5,000 third instars of codling moths in each of the two CATTS treatments of 45°C for 45 min and 47°C for 25 min under a 1% oxygen and 15% carbon dioxide,

Table 1. LT_{90} and LT_{95} with 95% confidence intervals for three embryonic stages of codling moth in sweet cherries subjected to CATTS treatments of 45°C under 1% O_2, 15% CO_2

Stage	*n*	LT_{90} (95% FL) (min)	LT_{95} (95% FL) (min)	Slope ± SE
White	2,558	15.5 (15.5–15.6)	18.9 (18.8–19.0)	4.35 ± 0.02
Red ring	2,380	13.1 (12.9–13.2)	19.0 (18.8–19.1)	2.25 ± 0.01
Blackhead	2,430	11.1 (10.9–11.2)	24.0 (23.7–24.4)	1.08 ± 0.01

All probits, $P < 0.0001$.

−2°C dew point environment with an air speed of 2 m/s were performed. Approximately 15 lb of mature Rainier sweet cherries were placed into 49.5 by 36.0 by 12.4 cm (length by width by depth) Rubbermaid containers. The fruit were infested with 500 third instars of codling moths. A layer of nylon organdy was secured over the top of the container with double stick tape, and then the lid was placed over the organdy. The fruit were placed under normal rearing conditions overnight before treatment. Untreated controls consisted of 10 lb of fruit infested with 200 third instars. Infested fruit were transferred into treatment lugs (described above) before treatment. Insects not in the fruit were removed and counted and subtracted from the total to give an actual number of insects in the fruit at time of treatment. Control fruit also were handled in the same manner. Treatments and evaluations were conducted as described previously with the exception that strict requirements on achieving target core temperatures were employed. Critical core target temperatures for the 45°C CATTS treatment were that core temperature of the fruit reach 42°C within 7–9 min and reach a final core temperature of 44.5°C in 22–24 min. Critical core target temperatures for the 47°C CATTS treatment were that fruit core temperatures reach 42°C within 7–9 min and reach a final core temperature of 45.5°C in 12–14 min (Fig. 3). Any treatments not achieving these requirements, or when the atmospheres did not meet minimum requirements (2% O_2, 10% CO_2) were not included in the study.

Oxygen and Carbon Dioxide Tests. The effects of varying levels of oxygen and carbon dioxide during CATTS treatments of fifth instar codling moth infesting sweet cherries was determined using chamber temperatures of 45 and 47°C and combinations of atmospheric environments of 0.5, 1.0, 6.0, 11.0, 16.0, and 20.0% oxygen and 0, 5, 10, 15, and 20% carbon dioxide. For each temperature and atmosphere combination, three time points were taken and mortality assessed. For the 45°C treatments, time points of 20, 30, and 40 min were taken. For the 47°C treatment, time points of 10, 15, and 20 min were taken. A group of 50 untreated larvae in sweet cherries served as controls for each day of testing. All CATTS treatments used in these studies were not optimized for achieving core target temperatures, as for the efficacy trials. This difference was due to one operator generally performing the treatments, and lug exchange times were increased, resulting in slightly longer heating times compared with the large-scale efficacy tests. Treatments and evaluations were conducted in the same manner as described previously.

Table 3. LT_{90} and LT_{95} with 95% confidence intervals for first through fifth instars of codling moth in sweet cherries subjected to CATTS treatments of 45°C under 1% O_2, 15% CO_2

Instar	*n*	LT_{90} (95% FL) (min)	LT_{95} (95% FL) (min)	Slope ± SE
First	709	34.9 (33.7–36.2)	56.5 (53.7–59.7)	1.73 ± 0.04
Second	637	18.2 (17.7–18.7)	26.5 (25.7–27.4)	2.23 ± 0.06
Third	698	33.6 (32.8–34.4)	47.0 (45.5–48.7)	2.49 ± 0.05
Fourth	757	48.7 (47.6–49.9)	60.6 (58.5–62.2)	3.92 ± 0.07
Fifth	485	35.9 (35.5–36.2)	40.0 (39.5–40.6)	7.60 ± 0.16

All probits, $P < 0.0001$.

Statistics. Probit analysis (PROC PROBIT) (SAS Institute 2000) was used to determine the most tolerant stage and to determining the LT_{50} and LT_{90} values in the oxygen/carbon dioxide series experiments. Analysis of covariance (ANCOVA) (SAS Institute 2000) also was used to determine interaction effects of the most tolerant stage experiments. Analysis of variance (ANOVA) and PROC GLM were used (SAS Institute 2000) to analyze the dose–response of third instars of codling moth to CATTS treatments. All mortality data were corrected for control mortality by using Abbott's equation (Abbott 1925) and then transformed using arcsine of the square root before statistical analyses.

Results and Discussion

Most Tolerant Stage. Comparison of the three embryonic stages of eggs and the five instars by using probit analysis indicated that all egg stages are less tolerant to CATTS treatments than all larval stages ($df = 1, P < 0.0001$) (Tables 1–4; Fig. 1). Of the three egg stages, the white stage was the most tolerant to

Table 2. LT_{90} and LT_{95} with 95% confidence intervals for three embryonic stages of codling moth in sweet cherries subjected to CATTS treatments of 47°C under 1% O_2, 15% CO_2

Stage	*n*	LT_{90} (95% FL) (min)	LT_{95} (95% FL) (min)	Slope ± SE
White	2,558	7.36 (7.2–7.5)	12.6 (12.4–12.7)	1.56 ± 0.02
Red ring	2,112	1.4 (1.1–1.7)	3.5 (3.1–3.9)	0.91 ± 0.04
Blackhead	2,514	***	***	***

All other probits, $P < 0.0001$.

*** Probit analysis could not be performed due to >95% mortality at first time point.

Table 4. LT_{90} and LT_{95} with 95% confidence intervals for first through fifth instars of codling moth in sweet cherries subjected to CATTS treatments of 47°C under 1% O_2, 15% CO_2

Instar	*n*	LT_{90} (95% FL) (min)	LT_{95} (95% FL) (min)	Slope ± SE
First	710	20.6 (20.2–20.9)	26.6 (26.0–27.3)	3.24 ± 0.07
Second	684	17.9 (17.6–18.3)	25.6 (24.8–26.6)	2.35 ± 0.07
Third	727	23.5 (22.9–23.9)	31.0 (30.1–32.1)	2.94 ± 0.07
Fourth	740	29.2 (28.5–30.2)	38.1 (36.9–39.5)	3.15 ± 0.06
Fifth	728	25.9 (25.2–26.6)	34.4 (33.2–35.7)	2.95 ± 0.07

All probits, $P < 0.0001$.

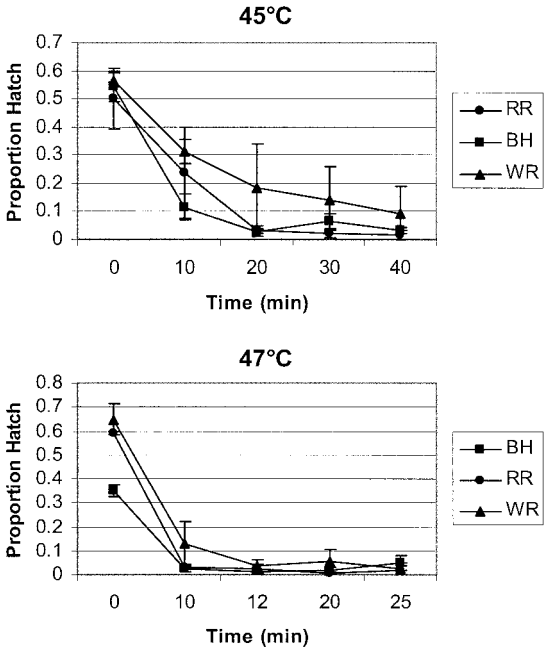

Fig. 1. Proportion of egg hatch of the three embryonic egg stages of codling moth after 45°C (A) and 47°C (B) under a 1% O_2, 15% CO_2 atmosphere, −2°C dew point and 2 m/s air speed CATTS treatments of sweet cherries. These treatments were not optimized for heating rate targets (i.e., time for core to reach 42°C was >10 min). White egg stage is indicated by ▲ (WR), red ring stage is indicated by ● (RR), and the blackhead stage is indicated by ■ (BH).

CATTS treatments (df = 1, $P < 0.0001$). The third and fourth instars are the most tolerant to CATTS (df = 1, $P < 0.0001$), but are approximately equal to each other in tolerance (Tables 3 and 4; Fig. 2). However, ANCOVA revealed that the egg stages are approximately equal to each other in tolerance (stage not significant, $P > 0.25$; stage*min interaction not significant for all but blackhead versus white at 45°C, $P = 0.279$). ANCOVA also showed that the fourth instar was more tolerant than second and third instars for the 45°C treatment (stage, $P < 0.0001$; stage*min, not significant) and that first, second, and third instars were approximately equal in tolerance ($P > 0.07$, stage*min not significant) and that the fifth instar was approximately equal in tolerance to first-third instars (stage $P < 0.001$, stage*min $P < 0.005$). For the 47°C treatment, ANCOVA showed that the fifth and fourth instars (stage, $P > 0.9988$) were approximately equal in tolerance. It also showed that the fifth instar was more tolerant than first and second instars (stage $P < 0.007$) and that the fourth instars were more tolerant than third and first instars ($P < 0.0049$), whereas the third instar was more tolerant than the second instar ($P < 0.026$), and first and second instars were approximately equal in tolerance ($P > 0.2709$).

Efficacy Tests. Third instars were used in the efficacy tests because current methyl bromide fumigation treatments against codling moth in sweet cherries destined to Japan are designed to control the third instar (Moffitt et al. 1992, Hansen et al. 2000), and this stage is relatively as tolerant to CATTS as the fourth instar. Also, it is generally recognized by both the United

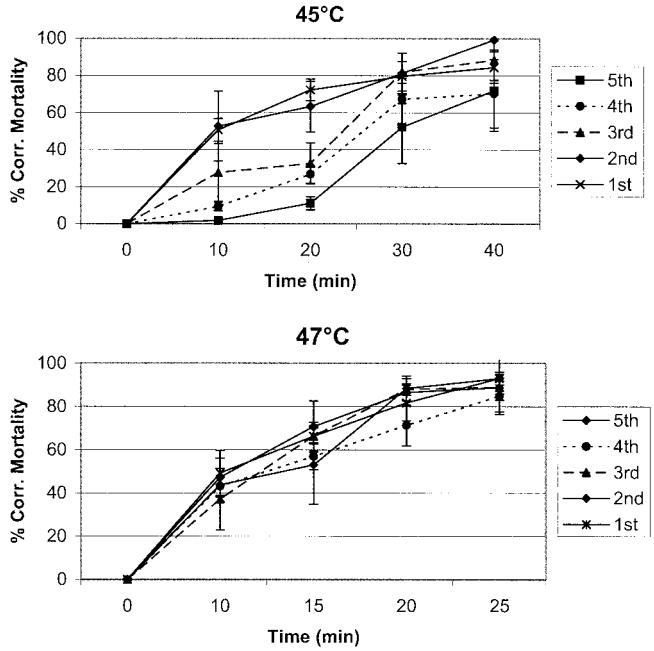

Fig. 2. Percentage of corrected mortality of the larval instars of codling moth to 45° (A) and 47°C (B) under a 1% O_2, 15% CO_2 atmosphere, −2°C dew point and 2 m/s air speed CATTS treatments of sweet cherries. These treatments were not optimized for heating rate targets (i.e., time for core to reach 42°C was >10 min).

Table 5. Results from CATTS efficacy tests of third instars of codling moth in sweet cherries

Treatment	Duration (min)	Control *n*	Control mortality (%)	Infested	Treated	% survivorship
45°C	45	1,395	9.2	6,000	5,076	0.0
47°C	25	1,100	9.8	6,901	5,759	0.0

Treatments of 45°C for 45 min and 47°C for 25 min under a 1% O_2, 15% CO_2 atmosphere with a −2°C dew point and 2.0 m/s air speed.

States and MAFF Japan that the third instar is the stage most likely to be in the fruit at time of harvest (Moffitt et al. 1992). Treatments at 45°C under a 1% O_2, 15% CO_2 atmosphere with −2°C dew point and 2 m/s air speed were used to treat 5,067 third instars with zero survivors (Table 5; Fig. 3). Treatments at 47°C under a 1% O_2, 15% CO_2 atmosphere with −2°C dew point and 2m/s air speed were used to treat 5,759 third instars with zero survivors (Table 5; Fig. 3). Average control mortality was <10% in all treatments. The number killed was adjusted from the control mortality and larval recovery (insects outside of fruit) during transfer from infesting bins to treatment lugs. These tests indicate that these treatments are efficacious for control of codling moth larvae in sweet cherries.

Oxygen and Carbon Dioxide Tests. The effects of low levels of oxygen on insect mortality are more pronounced than the levels of carbon dioxide (Table 6). Efficacious treatments were obtained for those having oxygen levels <6% (1 and 0.5% oxygen treatments) at both temperatures. The levels of carbon dioxide were less important in achieving mortality goals with the 45°C treatments than for the 47°C treatments. Levels of carbon dioxide between 10 and 20% were more effective than treatments under 0 and 5% CO_2 for the 47°C treatment (Table 6). The lower levels of effectiveness of the elevated CO_2 in the 47°C treatments than in the 45°C treatments may be due to the short duration of these treatments.

The combined effects of low oxygen and elevated carbon dioxide on insect mortality during CATTS treatments demonstrate two physiological principles. The first principle is that insects must have oxygen to support metabolism at elevated temperatures. The second principle is that high levels of carbon dioxide are toxic to insects and insect respiration is geared to control the release of carbon dioxide. However, we showed that insects can temporarily down-regulate respiration to a heat load (Neven 1998b). Most likely, the elevated carbon dioxide prevents the temporary down regulation, and forces the spiracles to remain open, compromising the oxygen balance needed to support increased metabolism at elevated temperatures. As with other published CATTS treatments (Neven and Mitcham 1996), we found that the combination effect of elevated temperatures in combination with the low oxygen, high carbon dioxide atmospheres had an additive effect on insect mortality during the treatment. The treatments described here differ from previous published CATTS treatments of sweet cherries (Neven and Mitcham 1996) in that we use a very rapid rate of heating, resulting in shorter

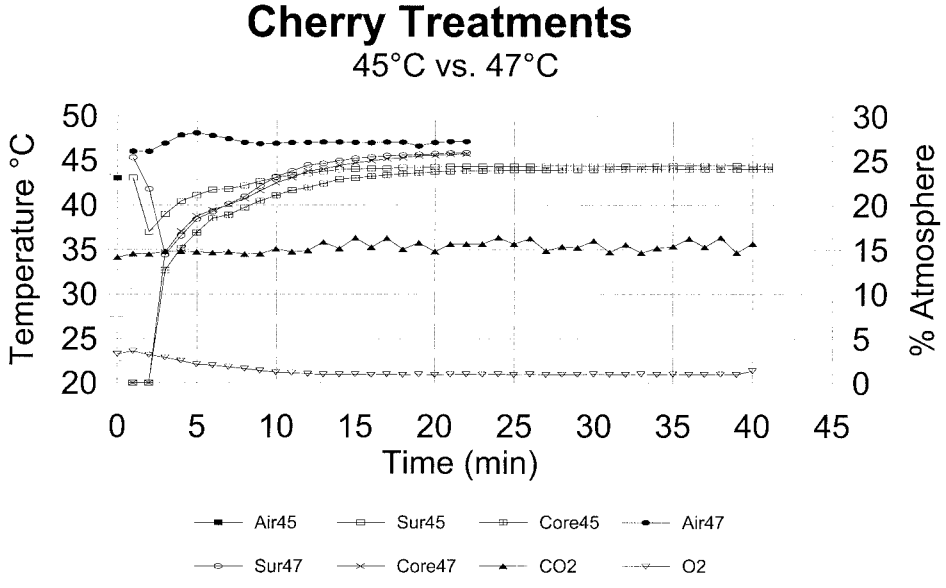

Fig. 3. Comparison of two CATTS treatment conditions for sweet cherries. One treatment is performed at a chamber temperature of 45°C (■) for a total of 45 min, whereas the other treatment is performed at a chamber temperature of 47°C (●) for a total of 25 min. Atmospheric conditions are indicated by (▲, CO_2; ▽, O_2). Fruit surface and core temperatures are denoted by □ surface at 45°C, ⊞ core at 45°C, ○ surface at 47°C, and x core at 47°C.

Table 6. Percentage of corrected mortality of third instars of codling moth infesting sweet cherries under varying levels of oxygen and carbon dioxide during CATTS treatments of 45 and 47°C chamber temperatures

CO_2	O_2	45°C			47°C		
		20 min	30 min	40 min	10 min	15 min	20 min
0	0.5	35.3	68.6	80.5	26.3	29.5	97.7
0	1	70.4	78.3	100	7.8	13.4	51.2
0	6	81.9	45.9	78.9	0.6	11.2	22.1
0	11	85.5	47.1	59.5	48.2	33	44.2
0	16	60.2	55.7	58.4	24.5	35.5	48.6
0	20	55.3	48.2	61.4	35.7	43.7	57.3
5	0.5	87.9	100	100	13.9	25.1	35.9
5	1	63.2	80.2	97.2	97.4	100	67.3
5	6	59.7	97.6	100	15.2	19.4	38.6
5	11	52.6	54.2	59.7	53.7	68.2	86.5
5	16	58.6	37.4	59.6	31.7	51.4	60.3
5	20	32.3	32.9	26.2	26.3	46.1	70.1
10	0.5	17.1	100	100	19.4	23.5	90.7
10	1	87.4	100	100	78.8	58.63	67.5
10	6	40.7	79.2	93.2	19.4	23.5	90.7
10	11	70.4	78.5	75.8	54.7	85.5	81.9
10	16	45.7	54.5	48.9	40.8	33	82.6
10	20	32.7	52.6	46.3	37.4	38.8	52.6
15	0.5	100	100	100	43.9	57.7	79.7
15	1	74.2	89.3	99.46	58.24	100	83.9
15	6	67.8	100	79.9	36.7	43.2	86.9
15	11	74.3	73.7	68.9	60.5	83.8	89.9
15	16	59.6	87.4	78.9	33.1	70.8	51.2
15	20	7.0	20.7	51.9	35.6	34.74	60.4
20	0.5	80.8	81.6	100	50.5	100	100
20	1	66.0	100	100	78.0	71.0	100
20	6	81.2	78.9	78.3	58.1	48.5	64.9
20	11	21.57	37.5	29.4	60.5	83.8	89.9
20	16	57.9	69.7	52.6	50.4	75.8	79.7
20	20	86.8	53.7	61.4	39.8	57.5	51.5

treatment times needed to achieve 100% mortality. We have shown that heating rate can have a dramatic effect on codling moth mortality (Neven 1998a). Therefore, we were able to shorten the treatment times for the 45°C CATTS and the 47°C CATTS treatments by 20 min as compared with previous research (Neven and Mitcham 1996).

These studies indicate that CATTS can be effective in achieving control of codling moth larvae in sweet cherries. Previous research has demonstrated that CATTS treatments of sweet cherries provides acceptable market quality with up to 3 wk of shelf life (Neven and Drake 1998, Neven and Drake 2000, Neven et al. 2001, Shellie et al. 2001). These treatments show promise as an alternative to methyl bromide fumigation for both conventional and organically grown sweet cherries.

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